II. Sampling and Testing Methods

A. Samples

The *Salmonella* isolates included in this report were recovered by FSIS from carcass rinsates (chicken), carcass swabs (turkey, cattle, and swine), and ground products (chicken, turkey, and beef).

Sampling methods used by FSIS for the PR/HACCP *Salmonella* verification testing program have changed since NARMS animal testing began. Before June of 2006, there were two phases of the FSIS regulatory program for *Salmonella* in raw products: non-targeted and targeted testing. Non-targeted samples were collected at establishments randomly selected from the population of eligible establishments, with a goal of scheduling every eligible establishment at least once a year. Targeted samples were collected from establishments that had a previously failed non-targeted sample set. Beginning in June of 2006, sampling was scheduled using risk-based criteria designed to focus FSIS resources on establishments with the most samples positive for *Salmonella* and the greatest number of samples with serotypes most frequently associated with human salmonellosis[1,2].

B. Isolation and Identification

Salmonella isolation from slaughter samples was conducted by FSIS at all three FSIS Regulatory Field Services Laboratories [Eastern (Athens, GA), Midwestern (St. Louis, MO) and Western (Alameda, CA)] following the "Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg" procedures as described in the Microbiology Laboratory Guidebook, section 4[3,4]. Each FSIS laboratory processes samples collected throughout the U.S. Isolates were forwarded by FSIS to the National Veterinary Services Laboratories, Ames, IA (NVSL) for serotyping. Serotype results were subsequently sent to the BEAR unit as they became available.

From 1998 to 2000, *Campylobacter* was isolated by all FSIS laboratories as part of the chicken monitoring baseline programs using the method described in the FSIS Microbiology Laboratory Guidebook[5]. Following presumptive identification, isolates were sent to BEAR for final confirmation and susceptibility testing as described below. Upon review of susceptibility data and isolation methods, it was determined that use of nalidixic acid as part of the culture selection criteria may have resulted in

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[1] USDA/FSIS. 2008. Serotypes Profile of Salmonella Isolates from Meat and Poultry Products. Available at http://www.fsis.usda.gov/Science/Serotypes_Profile_Salmonella_Isolates/index.asp.

[2] USDA/FSIS. FSIS Scheduling Criteria for Salmonella Sets in Raw Classes of Product. Available at http://www.fsis.usda.gov/PDF/Scheduling_Criteria_Salmonella_Sets.pdf.

[3] USDA/FSIS. 2004. Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products. Microbiological Lab Guidebook 4.03. Available at http://www.fsis.usda.gov/PDF/MLG_4_03.pdf.

[4] USDA/FSIS. 2010. Laboratories and Procedures. Available a.t http://www.fsis.usda.gov/Science/Laboratories_&_Procedures/index.asp.

[5] USDA/FSIS. 1998. Isolation, Identification, And Enumeration Of Campylobacter jejuni/coli From Meat And Poultry Products. Microbiology Laboratory Guidebook, chapter 6. Available at http://www.fsis.usda.gov/ophs/Microlab/Mlgchp6.pdf.

recovery of isolates more likely to be resistant to quinolones. A comparative study was initiated by BEAR in 2001.

For the first half of 2001, BEAR pilot tested several isolation methods for *Campylobacter* prior to adopting a new method in July which involved concentrating spent carcass rinsate and decanting the supernatant prior to culture of the pellet. Since that time, only rinsates from the FSIS Eastern Lab containing ≥ 10 ml have been used. Thus, all rinsates tested for *Salmonella* were not processed for *Campylobacter* or *E. coli*. Also important to note is that when the FSIS *Campylobacter* baseline testing ended, rinsates were no longer temperature controlled during shipment; this may affect recovery of *Campylobacter*. Final confirmation and speciation of *Campylobacter* isolates were obtained using the BAX® System Q7 (DuPont Qualicon; Wilmington, DE). This real-time PCR assay, able to detect *C. coli*, *C. jejuni*, and *C. lari*, was performed according to manufacturer's directions.

BEAR started isolating generic *E. coli* from these same rinsates in 2000. For *E. coli*, a sample of the rinsate was pre-enriched overnight before streaking onto a CHROMAgar™ ECC plate (DRG International; Mountainside, NJ). Plates were incubated at ±36° for 18-24 h as described by the manufacturer. Blue-green colonies, typical of generic *E. coli,* were selected for susceptibility testing and confirmed as *E. coli* using the Vitek (bioMérieux, Inc; Durham, NC).

C. Antimicrobial Susceptibility

In 2008, *Salmonella*, *Campylobacter*, and *E. coli* were tested using a semi-automated broth micro dilution system (Sensitire®, Trek Diagnostic Systems, Inc., Westlake, Ohio) and a custom made 96 well panel of antimicrobials (catalog no. CMV1AGNF for *Salmonella* and *E. coli;* catalog no. CAMPY for *Campylobacter*) to determine the minimum inhibitory concentration (MIC) of antimicrobials important in both human and veterinary medicine. Tables 1 and 2 list the antimicrobials tested, including the breakpoints for *Salmonella*/*E. coli* and *Campylobacter*, respectively. From 1998-2004, MICs for *Campylobacter* isolates were determined using Etest® (AB Biodisk; Solna, Sweden) as per manufacturer's direction with the exception that MICs were not rounded up prior to categorization. In 2005, the animal arm of NARMS switched to using the Sensititre® broth microdilution system for *Campylobacter* although the antimicrobials tested as described above for *Salmonella* and *E. coli* differed (Table 2) Regardless of the susceptibility testing method used, antimicrobial resistance was determined using Clinical and Laboratory Standards Institute (CLSI) breakpoints, when available[6,7,8].

In January 2010, CLSI published new MIC breakpoints for several cephalosporin antimicrobials for Enterobacteriaceae[9]. In particular, the resistance breakpoint for ceftriaxone changed (decreased) from ≥ 64 µg/ml to ≥ 4 µg/ml. In this report, the revised breakpoints for ceftriaxone are used and have been

[6] CLSI. 2006. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. CLSI document M45-A. CLSI, Wayne, PA.
[7] CLSI. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Third Edition. CLSI document M31-A3. CLSI, Wayne, PA.
[8] CLSI. 2009. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S19. CLSI, Wayne, PA.
[9] CLSI. 2010. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. CLSI, Wayne, PA.

retrospectively applied to data from previous years; therefore, ceftriaxone resistance in previous reporting years will differ from what is presented in this report. It is important to note that the actual raw data has not changed over time, only the way that it is interpreted. For antimicrobial agents without CLSI approved breakpoints, interpretive criteria as established by the NARMS working group were used.

Quality control strains used for *Salmonella* and *E. coli* susceptibility testing included *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, Pseudomonas aeruginosa ATCC 27853 and *Staphylococcus aureus* ATCC 29213. *Campylobacter jejuni* ATCC 33560 was used for *Campylobacter* susceptibility testing.

Table 1. *Salmonella* and *E. coli* Interpretive Criteria (breakpoints)[10]

CLSI Antimicrobial Class[11]	Antimicrobial Agent	Breakpoints (µg/ml)		
		Susceptible	Intermediate	Resistant
Aminoglycosides	Amikacin	≤ 16	32	≥ 64
	Gentamicin	≤ 4	8	≥ 16
	Kanamycin	≤ 16	32	≥ 64
	Streptomycin[12]	≤ 32	Not Applicable	≥ 64
β-Lactam/β-Lactamase Inhibitor Combinations	Amoxicillin–Clavulanic Acid	≤ 8 / 4	16/8	≥ 32 / 16
Cephems	Cefoxitin	≤ 8	16	≥ 32
	Ceftiofur	≤ 2	4	≥ 8
	Ceftriaxone[13]	≤ 8	16 - 32	≥ 64
	Cephalothin	≤ 8	16	≥ 32
Folate Pathway Inhibitors	Sulfonamides[14]	≤ 256	Not Applicable	≥ 512
	Trimethoprim–Sulfamethoxazole	≤ 2 / 38	Not Applicable	≥ 4 / 76
Penicillins	Ampicillin	≤ 8	16	≥ 32
Phenicols	Chloramphenicol	≤ 8	16	≥ 32
Quinolones	Ciprofloxacin	≤ 1	2	≥ 4
	Nalidixic acid	≤ 16	Not Applicable	≥ 32
Tetracyclines	Tetracycline	≤ 4	8	≥ 16

[10] Breakpoints established by CLSI (Clinical and Laboratory Standards Institute) were used when available
[11] According to CLSI M100 document
[12] There are no CLSI breakpoints for streptomycin
[13] In this report, the revised ceftriaxone breakpoints from the CLSI M100-S20 document, published in January 2010, were used (≥ 4 µg/ml). In previous NARMS reports the ceftriaxone breakpoints from the CLSI M100-S19 were used (≥ 64 µg/ml)
[14] From 1997 through 2003, sulfamethoxazole was tested. Sulfisoxazole replaced sulfamethoxazole beginning in 2004

Table 2. *Campylobacter* Interpretive Criteria (breakpoints)[15]

CLSI Antimicrobial Class[16]	Antimicrobial Agent	Breakpoints (µg/ml) Etest (1998-2004)			Breakpoints (µg/ml) Broth Microdilution (2005-2008)		
		Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin	≤ 4	8	≥ 16	≤ 2	4	≥ 8
Lincosamides	Clindamycin	≤ 0.5	1 - 2	≥ 4	≤ 2	4	≥ 8
Macrolides	Azithromycin	≤ 0.25	0.5 - 1	≥ 2	≤ 2	4	≥ 8
	Erythromycin	≤ 0.5	1 - 4	≥ 8	≤ 8	16	≥ 32
Ketolides	Telithromycin	Not Tested	Not Tested	Not Tested	≤ 4	8	≥ 16
Phenicols	Florfenicol	Not Tested	Not Tested	Not Tested	≤ 4	Not Applicable	Not Applicable
	Chloramphenicol	≤ 8	16	≥ 32	Not Tested	Not Tested	Not Tested
Fluoroquinolones	Ciprofloxacin	≤ 1	2	≥ 4	≤ 1	2	≥ 4
Quinolones	Nalidixic acid	≤ 16	Not Applicable	≥ 32	≤ 16	32	≥ 64
Tetracyclines	Tetracycline	≤ 4	8	≥ 16	≤ 4	8	≥ 16

[15] Breakpoints established by CLSI (Clinical and Laboratory Standards Institute) were used when available. CLSI breakpoints are available only for erythromycin, ciprofloxacin, and tetracycline

[16] According to CLSI M100 document

D. Phage Typing

Salmonella Typhimurium and *S.* Typhimurium variant 5- isolates with resistance to at least ampicillin, chloramphenicol, sulfisoxazole and tetracycline (ACSuT) were submitted to NVSL for phage typing.